

Please replace the paragraph beginning at page 4, line 35, with the following rewritten paragraph:

A2 Figure 2 shows the nucleotide sequences of exon 1 of the mouse (SEQ.ID.NO.: 5) and human (SEQ.ID.NO.: 6) Fgl2 genes;†

Please replace the paragraph beginning at page 4, line 37, with the following rewritten paragraph:

A3 Figure 3 shows the nucleotide sequences of exon 2 of the mouse (SEQ.ID.NO.: 7) and human (SEQ.ID.NO.: 8) Fgl2 genes;†

Please replace the paragraph beginning at page 5, line 1, with the following rewritten paragraph:

A4 Figure 4 shows the nucleotide sequence of the 3' UTR of hFgl2 (SEQ.ID.NO.: 9);†

Please replace the paragraph beginning at page 5, line 2, with the following rewritten paragraph:

A5 Figure 5 shows the amino acid sequences of the mouse (SEQ.ID.NO.: 4) and human (SEQ.ID.NO.: 2) Fgl2 proteins with the serine protease sites boxed;†

Please replace the paragraph beginning at page 5, line 7, with the following rewritten paragraph:

A6 Figure 8 shows the nucleotide sequence of the mouse (SEQ.ID.NO.: 10) and human (SEQ.ID.NO.: 11) Fgl2 gene promoter regions;†

Please replace the paragraph beginning at page 5, line 9, with the following rewritten paragraph:

A7 Figure 9 shows the nucleic acid sequence of the transcription binding sites in the putative promoter region of *hfgl2* (SEQ.ID.NO.: 12);†

Please replace the paragraph beginning at page 5, line 22, with the following rewritten paragraph:

A₈ Figure 17 shows the Fgl-2 promoter DNA sequence (SEQ.ID.NO.: 13). #

Please replace the paragraph beginning at page 7, line 7, with the following rewritten paragraph:

A₉ The present invention also provides an antibody that binds an epitope of hFgl2 comprising the amino acids at positions 300 to 400 in Figure 5. In a preferred embodiment, the present invention provides an antibody that binds an epitope of hFgl2 comprising the amino acids at positions 364-378 (DRYPSGNCGLYYSSG) (SEQ.ID.NO.: 18) in Figure 5. #

Please replace the paragraph beginning at page 18, line 6, with the following rewritten paragraph:

A₁₀ As hereinbefore mentioned, the present inventor has cloned and sequenced genomic hFgl2. In this regard, the entire genomic sequence as well as the sequence of the promoter region, shown in Figure 8 (SEQ.ID.NO.: 11), and the 3' UTR, shown in Figure 4 (SEQ.ID.NO.: 9), are included within the scope of the invention. #

Please replace the paragraph beginning at page 18, line 10, with the following rewritten paragraph:

A₁₁ Accordingly, in one embodiment the present invention provides an isolated nucleic acid molecule comprising (a) the sequence shown in Figure 8 (SEQ.ID.NO.: 11), where T can also be U; (b) nucleic acid sequences which have substantial sequence identity with (a); and (c) a fragment of (a) or (b). #

Please replace the paragraph beginning at page 18, line 14, with the following rewritten paragraph:

A₁₂ In another embodiment the present invention provides an isolated nucleic acid molecule comprising (a) the sequence shown in Figure 4 (SEQ.ID.NO.: 9), where T can also be U; (b) nucleic acid sequences which have substantial sequence identity with (a); and (c) a fragment of (a) or (b). #

Please replace the paragraph beginning at page 18, line 18, with the following rewritten paragraph:

A₁₃ The present invention also includes fragments of the nucleic acid sequences shown in Figure 2 or 3 or SEQ.ID.NOS.: 6 or 8 which have particular utility in the methods and compositions described above. The fragments generally comprise a nucleic acid sequence having at least 15 bases which will hybridize to the sequences shown in Figures 2 and 3 or SEQ.ID.NOS.: 6 or 8 under stringent hybridization conditions. #

Please replace the paragraph beginning at page 19, line 7, with the following rewritten paragraph:

A14 Further, it will be appreciated that the invention includes nucleic acid molecules comprising nucleic acid sequences having substantial sequence identity with the nucleic acid sequences shown in Figures 2 (SEQ.ID.NO.: 6), 4 (SEQ.ID.NO.: 9) and 8 (SEQ.ID.NO.: 11) and fragments thereof having at least 15 bases which will hybridize to these sequences under stringent hybridization conditions. The term "sequences having substantial sequence identity" means those nucleic acid sequences which have slight or inconsequential sequence variations from the sequences disclosed in Figures 2 (SEQ.ID.NO.: 6) and 3 (SEQ.ID.NO.: 8), i.e. the sequences function in substantially the same manner to produce substantially the same activity as described herein for Fgl2. The variations may be attributable to local mutations or structural modifications. Nucleic acid sequences having substantial identity include nucleic acid sequences having at least 72%, preferably at least 75-95% identity with the nucleic acid sequences as shown in Figure 2 (SEQ.ID.NO.: 6) and Figure 3 (SEQ.ID.NO.: 8). #

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Please replace the paragraph beginning at page 19, line 18, with the following rewritten paragraph:

A15 Isolated and purified nucleic acid molecules encoding a protein having the activity of human Fgl2 as described herein, and having a sequence which differs from the nucleic acid sequence shown in Figure 2 (SEQ.ID.NO.: 6) and Figure 3 (SEQ.ID.NO.: 8) due to degeneracy in the genetic code are also within the scope of the invention. Such nucleic acids encode functionally equivalent proteins (e.g., a protein having human Fgl2 prothrombinase activity) but differ in sequence from the sequence of Figure 2 (SEQ.ID.NO.: 6) and Figure 3 (SEQ.ID.NO.: 8) due to degeneracy in the genetic code. #

Please replace the paragraph beginning at page 19, line 32, with the following rewritten paragraph:

A16 --The nucleic acid molecules of the invention can be used to isolate an Fgl2 from other species. For example, a labelled nucleic acid probe based on all or part of the nucleic acid sequence shown in Figure 2 (SEQ.ID.NO.: 6) and 3 (SEQ.ID.NO.: 8) can be prepared, and used to screen an appropriate DNA library (e.g. a cDNA or genomic DNA library). Nucleic acids isolated by screening of a cDNA or genomic DNA library can be sequenced by standard techniques.--

Please replace the paragraph beginning at page 20, line 19, with the following rewritten paragraph:

A17 The initiation codon and untranslated sequences of human Fgl2 may be determined using currently available computer software designed for the purpose, (e.g. PC/Gene (IntelliGenetics Inc., Calif.)). The nucleic acid sequence for a 3' untranslated region of hfgl2 is shown in Figure 4 (SEQ.ID.NO.: 9). The intron-exon structure and the transcription regulatory sequences of the gene encoding human Fgl2 may be identified by using a nucleic acid molecule of the invention encoding human Fgl2 to probe a genomic DNA clone library. Regulatory elements can be identified using conventional techniques. The function of the elements can be confirmed by using them to express a reporter gene such as the bacterial gene lacZ which is operatively linked to the elements. These constructs may be introduced into cultured cells using standard procedures or into non-human transgenic animal models. Such constructs may also be used to identify nuclear proteins interacting with the elements, using techniques known in the art.--7

Please replace the paragraph beginning at page 20, line 31, with the following rewritten paragraph:

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A18 In addition to the full length amino acid sequence (Figure 5), the proteins of the present invention include truncations and analogs, and homologs of the protein and truncations thereof as described herein. A truncated Fgl2 protein or fragment of the human Fgl2 protein is a portion of the full-length Fgl2 amino acid sequence having one or more amino acid residues deleted. The deleted amino acid residue(s) may occur anywhere in the polypeptide, including at either the N-terminal or C-terminal end or internally. Fgl2 fragments typically will have a consecutive sequence of at least 10, 15, 20, 25, 30, or 40 amino acid residues that are identical to the sequences of the human Fgl2. The truncations or portions of the Fgl2 protein may comprise an antigenic site that is capable of cross-reacting with antibodies raised against the Fgl2 protein whose sequence is shown in Figure 5 (SEQ.ID.NOS: 2 and 4). Therefore, immunogenic portions or fragments of human Fgl2 proteins are within the scope of the invention (e.g. amino acids 300 to 400 in Figure 5). Preferably the truncated protein or portion of the protein binds with an affinity of at least about 10^6 L/mole to an antibody raised against human Fgl2. ##

Please replace the paragraph beginning at page 21, line 14, with the following rewritten paragraph:

A19 --The proteins of the invention may also include analogs of human Fgl2 as shown in Figure 5 (SEQ.ID.NOS: 2 and 4) and/or truncations thereof as described herein, containing one or more amino acid substitutions, insertions, and/or deletions. Amino acid substitutions may be of a conserved or non-conserved nature. Conserved amino acid substitutions involve replacing one or more amino acids with amino acids of similar charge, size, and/or hydrophobicity characteristics. When only conserved substitutions are made the resulting analog should be functionally equivalent to human Fgl2 as described herein. Non-conserved substitutions involve replacing one

A29 or more amino acids with one or more amino acids which possess dissimilar charge, size, and/or hydrophobicity characteristics.--

Please replace the paragraph beginning at page 21, line 23, with the following rewritten paragraph:

A20 ~~One~~ One or more amino acid insertions may be introduced into the amino acid sequence as shown in Figure 5 (SEQ.ID.NOS: 2 and 4). Amino acid insertions may consist of single amino acid residues or sequential amino acids ranging from 2 to 15 amino acids in length. For example, amino acid insertions may be used to destroy the prothrombinase activity of the protein.

Please replace the paragraph beginning at page 21, line 27, with the following rewritten paragraph:

A21 Deletions may consist of the removal of one or more amino acids, or discrete portions (e.g. amino acids) from the human Fgl2 amino acid sequence as shown in Figure 5 (SEQ.ID.NOS: 2 and 4). The deleted amino acids may or may not be contiguous. The lower limit length of the resulting analog with a deletion mutation is about 10 amino acids, preferably 100 amino acids. ~~2~~

Please replace the paragraph beginning at page 21, line 32, with the following rewritten paragraph:

A22 The proteins of the invention also include homologs of human Fgl2 as shown in Figure 5 (SEQ.ID.NOS: 2 and 4) and/or truncations thereof as described herein. Such homologs are proteins whose amino acid sequences are comprised of the amino acid sequences of human Fgl2 regions from other species that hybridize under stringent hybridization conditions (see discussion of stringent hybridization conditions herein) with a probe used to obtain human Fgl2 as shown in Figure 5 (SEQ.ID.NOS: 2 and 4). It is anticipated that a protein comprising an amino acid sequence which is at least 72% preferably 75 to 90% similar, with the amino acid sequence shown in Figure 5 (SEQ.ID.NOS: 2 and 4) will exhibit prothrombinase activity. ~~4~~

Please replace the paragraph beginning at page 23, line 36, with the following rewritten paragraph:

A23 Human genomic DNA from the liver was amplified by Polymerase Chain Reaction using primers specific to the human cDNA sequence obtained from GenBank, that corresponds to exon 2 of mouse fgl2 gene; the sense primer CAA AAG AAG CAG TGA GAC CTA CA (SEQ.ID.NO.: 14) (hufpl7) is at position 692, and the antisense primer TTA TCT GGA GTG GTG AAA AAC TT (SEQ.ID.NO.: 15) (hufpl8) is at position 1133 of the human cDNA. The PAC library, from Genome Systems Inc. (St. Louis, Missouri), was screened using the single amplicon, of about 300 nucleotides

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correl

in length, that was produced from the above Polymerase Chain Reaction. Three clones, namely 6359, 6360, and 6361 were found positive for this screening. The plasmids containing these three clones were purified using the Qiagen maxiprep DNA purification protocol. The quality of the purified DNA and the presence of the inserts were verified by digesting the plasmid with Not 1 restriction enzyme (Canadian Life, Burlington, Canada), and subjecting the samples to Clamped Homogenous Electric Field (CHEF) gel electrophoresis, at 120 angle, 6 Volts, 1-20 seconds ramp interval, 0.5X TBE, and run time of 18 hours.

Please replace the paragraph beginning at page 24, line 13, with the following rewritten paragraph:

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A24

The clone 6360 was chosen for the rest of the study because in a dot blot analysis it consistently hybridized to sense primer GCA AAC AAT GAA ACA GAG GAA A (SEQ.ID.NO.: 16) (huflp1) at position 100 and anti-sense primer at position ATT GCC CTA TTA GAT AAC GAA TAC (SEQ.ID.NO.: 17) (huflp2) at position 1400. In order to reduce the DNA into fragments of 5 to 10 kb, which is a convenient size range to work with, the 6360 clone was digested under sub-optimal conditions with the restriction enzyme Sau 3A (Canadian Life, Burlington, Canada). The appropriate digest condition was found by incubating 5 µg of DNA with 1 µl of 2 µ/µl, 0.5 µ/µl, and 0.1 µ/µl of Sau 3A for one hour, at 37°C in a total reaction volume of 20 µl and observing the size range of the DNA fragments on a CHEF gel; the run conditions are 1 to 10 seconds ramp interval, 4.5 volts, 120 angle, 0.5X TBE, and a run time of 16 hours. The 6360 clone was large scale restriction digested by proportionately increasing the amount of DNA, reaction volume, and the amount of enzyme, that is, 10 µg, 40 µl, and 2 µl respectively. The final products of the restriction digest were subjected to CHEF gel electrophoresis at the above conditions. The DNA band corresponding to 6-9kb was excised and fragments were extracted using the Gene Clean DNA purification kit (Bio/Can Scientific, Mississauga, Ontario). The fragments were ligated into the alkaline phosphatase (Pharmacia, Uppsala, Sweden) treated BamH1 site of the Bluescript II vector (Stratagene) and transfected into DH10B competent cells by electroporation. This Bluescript II library was screened using the huflp1 and 2 primers. The primers were labeled at the 5 end with gamma P32 by using the enzyme Polynucleotide Kinase (Pharmacia, Uppsala, Sweden); these primers were used to screen the Bluescript library. The clone J14 hybridized to both these primers and was used for the subsequent work.

Please replace the paragraph beginning at page 27, line 24, with the following rewritten paragraph:

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--An AP1 site is located about 20 nucleotides from the TATA box (Figures 8 and 9). The consensus for AP1 motif is TGASTCA (SEQ.ID.NO.: 19), where S is a guanine or a cytosine. Except for the central S, cytosine in humans and guanine in mouse, the AP1 site is identical in mouse and human direct prothrombinase genes. AP1 is

Please replace the paragraph beginning at page 33, line 25, with the following rewritten paragraph:

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Please replace Table 3 beginning at page 55 with the following amended Table 3:

A27

TCF20: 2520000

PRIMER	SEQUENCE	5' POSITION	tm 2(A+T)+4(G+C)
HUFLP1	GCA AAC AAT GAA ACA GAG GAA A (SEQ.ID.NO.: 16)	100	60
HUFLP2	ATT GCC CTA TTA GAT AAC GAA TAC (SEQ.ID.NO.: 17)	1399	64
HUFLP3	AAC GGA GAC CCA GGC AGA AAC (SEQ.ID.NO.: 27)	349	66
HUFLP4	CTT CGG GAG CTG AAT AGT CAA (SEQ.ID.NO.: 28)	243	62
HUFLP5	GAC AGC AAA GTG GCA AAT CTA (SEQ.ID.NO.: 29)	553	60
HUFLP6	TTC TGG TGA AGT TGG TGC TCC (SEQ.ID.NO.: 30)	832	64
HUFLP7	CAA AAG AAG CAG TGA GAC CTA CA (SEQ.ID.NO.: 31)	693	66
HUFLP8	TTA TCT GGA GTG GTG AAA AAC TT (SEQ.ID.NO.: 15)	1125	62
HUFLP9	TGA CCA AGA GTA AGG AAA TGA (SEQ.ID.NO.: 32)	908	58
HUFLP10	TGA CTG TAT TTG TTC TTG GCT G (SEQ.ID.NO.: 33)	639	62
HUFLP11	TTC TGG GAA CTG TGG GCT GTA (SEQ.ID.NO.: 34)	1134	64
HUFLP12	CCA GCT TCA TCT TTA CAG T (SEQ.ID.NO.: 35)	43	54
HUFLP13	AAT CAC TCT GTT CAT TCC TCC (SEQ.ID.NO.: 36)	1353	60
HUFLP14	GAA ATA ATA TGC ATT GAA A (SEQ.ID.NO.: 37)	-173	36
HUFLP14R	AAC GCA CAG GAA GAG GAG A (SEQ.ID.NO.: 38)	-96	58
HUFLP15	TTG ACA TCC TTT GAG ATA T (SEQ.ID.NO.: 39)	1459??	50
HUFLP16	ATG GGG CAT TGG GGA GC (SEQ.ID.NO.: 40)	-427	56
HUFLP17	GGC TAT CTC CTC TTC CTG T (SEQ.ID.NO.: 41)	-118	58
HUFLP18	TGA GCT ATG CCA GTG TCT GT (SEQ.ID.NO.: 42)	-755	60
HUFLP19	CAA GCG TAG TAT ACC AAA T (SEQ.ID.NO.: 43)	-288	52
HUFLP20	AAG GCA GGA AAG AGG AAC (SEQ.ID.NO.: 44)	-961	54
HUFLP21	GAC AAA GGA ATA GAA AGT AGC (SEQ.ID.NO.: 45)	-601	58
HUFLP22	CAG GGC AAA AAT CTA AAT G (SEQ.ID.NO.: 46)	-1092	52
HUFLP23	GCC CAG AGA GCA GGT AGA A (SEQ.ID.NO.: 47)	-883	60
HUFLP24	CCA GCC AGG GTT GAA ATA (SEQ.ID.NO.: 48)	3' end	54
HUFLP25	GCC CTG TCA GTC ATT TTG (SEQ.ID.NO.: 49)	promoter: not used	54
HUFLP26	AAA AAC CTA CCA GTA GTC T (SEQ.ID.NO.: 50)	3' end	52
HUFLP28	TTG GGG TGA CAT TAT GC (SEQ.ID.NO.: 51)	2399	50
HUFLP 29	TGA GCA GCA CTG TAA AGA TG (SEQ.ID.NO.: 52)	16	58
HUFLP30	GTG GCT TAA AGT GCT TGG GT (SEQ.ID.NO.: 53)	1350	60